PHAGOCYTOSIS, WITH PARTICULAR REFERENCE TO ENCAPSULATED BACTERIA

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The phagocytic cells of the mammalian host provide a remarkable defense against a wide variety of bacteria, fungi, and protozoa. Whether they play a protective role in infections caused by viruses and rickettsiae is at present a matter of conjecture. The discovery of "pinocytosis" (Gey, 1954), however, and recent observations relating to bacterial endotoxins (Berthrong and Cluff, 1953; Braude *et al.*, 1955; Collins and Wood, 1959), suggest that a mechanism closely analogous to phagocytosis may aid in the disposal of submicroscopic parasites and even of toxins of relatively small molecular dimensions.

In the case of bacterial disease the process of phagocytosis *per se* may or may not be a critical factor in determining the ultimate fate of the parasite. Manifestly, the outcome of each encounter between microbe and phagocyte depends upon: (a) whether or not the microbe is promptly ingested and (b) whether, once ingested, it is able to survive.

Most bacteria are readily ingested by both polymorphonuclear leucocytes and macrophages (Suter, 1956). Their pathogenicity is not due to resistance to phagocytosis, but rather to properties which enable them to thrive in intraphagocytic sites. These properties are as yet poorly defined (Hirsch, 1958) and will probably remain obscure until more is learned about the precise mechanisms by which phagocytic cells kill bacteria. Other papers in the present symposium discuss this highly important aspect of "cellular immunity."

Although phagocytosis, *per se,* may not lead to the destruction of a microorganism, it may nevertheless curtail its spread. Furthermore, it may protect it against potentially lethal factors in the environment, including antibodies (Rous and Jones, 1916) and antimicrobial drugs (Magoffin and Spink, 1951; Mackaness, 1952; Shaffer *et al.*, 1953*a, b*). Thus the phagocytic process may influence the course of an infection without being highly bactericidal.

A number of bacterial species important to man, on the other hand, are promptly killed by phagocytic cells, once they have been ingested (Wood, 1951). They owe their virulence primarily to surface components, often demonstrable as capsules, which allow them to resist phagocytosis. They include *Diplococcus pneumoniae* (MacLeod, 1958*a*), *Streptococcus pyogenes* (McCarty, 1958), *Klebsiella pneumoniae* (MacLeod, 1958*b*), and *Pasteurella pestis* (Burrows, 1955). The recent findings of Cohn and Morse (1959) suggest that *Staphylococcus aureus* possesses similar properties. As perhaps might be expected, such bacteria, which behave essentially as extracellular parasites, tend to produce relatively acute infections, whereas those capable of surviving within phagocytes are more likely to cause chronic infections (Wood, 1951).

The following discussion will be limited to encapsulated bacteria that are readily killed by phagocytic cells. In the diseases they produce, phagocytosis is the principal defense mechanism which protects the host (Wood, 1951; Florey, 1958). A comprehensive treatment of phagocytosis in general will be found in the excellent review of Suter (1956).

Metchnikoff's theory that bacteria are destroyed by phagocytes first met with a cold reception when advanced in the late 1880's (Metchnikoff, 1887). Understandably impressed by Buchner's demonstration of bactericidal substances in serum (Buchner, 1890) and by von Behring's discovery of both tetanus and diphtheria antitoxins (von Behring and Kitasato, 1890), pathologists of the day believed that resistance to bacterial infections depended entirely upon humoral factors in the serum. Thus there arose in the ensuing decades a spirited controversy between the great majority of investigators, who adhered to the doctrine of humoral immunity, and the few followers of Metchnikoff, who defended the theory of cellular immunity (Bulloch, 1938).

The discovery of opsonins by Wright and Douglas (1903) partly reconciled the two con-

flicting theories, by proving that under certain circumstances antibodies and phagocytes may play a joint role in destroying bacteria. Nevertheless, antibodies remained in the ascendancy, for they could be easily measured in the laboratory (Dubos, 1945a).

Furthermore, several important species of bacteria (including pneumococci, hemolytic streptococci, and Friedländer's bacilli) were found to possess capsules, which appeared to protect them against phagocytosis, except when they had been previously opsonized with specific antibody (Dubos, 1945b). This important observation led to the immunological dictum that fully encapsulated bacteria could not be phagocytized except in the presence of opsonins (Zinsser et al., 1939). This dictum, which in a sense recognized only specific immunity, has proved to be untenable.

Except when antibodies are injected in the form of antiserum, they are acquired at a relatively slow rate. Not for many hours, or even several days after the host is infected, can they be detected either in the tissues or in the blood[1] (Wood et al., 1946a; Sale et al., 1947). What then prevents overwhelming microbial invasion from occurring during the pre-antibody phase of acute infections caused by encapsulated bacteria? The answer lies in a nonspecific immune mechanism which, for want of a better name, we have called surface phagocytosis (Wood et al., 1946b).

When encapsulated bacteria, such as pneumococci or Friedländer's bacilli, are incubated without antibody on a glass slide, phagocytosis fails to occur, despite the fact that the motile leucocytes frequently come into direct contact with the organisms (Wood et al., 1946b; Smith and Wood, 1947). If antibody, on the other hand, is added to such a preparation, the opsonized bacteria are readily ingested (Wood et al., 1946b). These are the laboratory observations which gave rise to the dictum mentioned previously.

In the living host, however, the situation is very different. For example, within a few hours after Friedländer's bacilli have been injected into the lung of a rat, phagocytosis is easily demonstrable in the infected alveoli (Sale and Wood, 1947).

Similarly, when pneumococci are introduced into the footpad (figure 1[2]), a marked phagocytic reaction occurs as soon as the leucocytes reach the site of the lesion (Smith and Wood, 1949b). In neither instance can antibodies be detected either in the tissues or in the circulation. Clearly, in the glass-slide preparations, some factor is missing which allows phagocytosis to take place in the tissues of the host.

The nature of this critical factor becomes evident as soon as one alters the conditions of the tests in vitro to make them simulate more closely those which obtain in vivo. If, for example, a phagocytic mixture (figure 2), which is negative on glass (left) is transferred to the surface of freshly excised tissue, or even to such an inert surface as that of moistened filter paper (right), phagocytosis promptly ensues. The process by which the leucocytes ingest the encapsulated organisms can be demonstrated by incubating the mixture in a thin section of formalin-fixed lung, where the behavior of the cells can be directly observed under the microscope. Here the leucocytes can be seen to phagocytize the encapsulated bacteria by trapping them against the alveolar walls (figure 3). Since the immovable surface of the tissues is used in this process, the resulting reaction has been called surface phagocytosis (Wood et al., 1946b).

Further study of the phenomenon has revealed that, whenever leucocytes are packed closely together, as in a dense exudate, they use each other's surfaces in trapping the organisms (Wood and Smith, 1947). Phagocytosis accomplished by this means is called intercellular surface phagocytosis.

Finally, if the organisms are caught in the interstices of a fibrin clot, they can be similarly phagocytized in the absence of antibody (Smith and Wood, 1949a).

Each of these three mechanisms of surface phagocytosis can be demonstrated with monocytes as well as with granulocytes (Sawyer et al., 1954). Each has also been directly observed in vivo (Wood et al., 1951).

Recently, the study of surface phagocytosis has been extended to infections caused by group A hemolytic streptococci (Foley et al., 1959; Foley

[1] Although there is suggestive evidence that antibody production may begin almost immediately (Stevens and McKenna, 1958), no data have been obtained to support the concept that amounts sufficient to opsonize encapsulated bacteria accumulate in vivo within less than 24 to 72 hr.

[2] Permission to reprint the photomicrographs and tables included in this review has been kindly granted by the Editors of the Journal of Experimental Medicine.

and Wood, 1959). Four strains of type 14 streptococci, which were known to vary considerably in intraperitoneal virulence, were selected for study. Their comparative properties are summarized in table 1.

The first two strains, referred to as S23, were derived from the same parent culture. Strain S23M produced a large amount of M protein (as indicated in the fourth column of the table) and was the most virulent for mice and rats. Strain S23G produced no detectable M substance and was relatively avirulent. The third and fourth strains, designated T14, were both M producers. The third, T14/46, was only slightly less virulent than S23M. The fourth, T14, produced less M protein than the other two M+ strains, and was the least virulent of all. As is indicated in the third column, labeled "size of capsule," the capsular envelopes of the S23 strains were somewhat larger than those of the T14 strains.

Besides being subjected to the usual phagocytic tests on glass and in glass roller tubes, the two S23 strains were also presented to leucocytes incubated on freshly excised tissues and on moistened filter paper. The latter methods were used to test for surface phagocytosis. Serum was excluded from all preparations in order to avoid the possible introduction of opsonins.

As shown in table 2, phagocytic tests performed with rat leucocytes on glass slides and in glass roller tubes failed to differentiate between the highly virulent S23M strain and its much less virulent relative, S23G. In contrast, the marked difference in susceptibility of the two strains to surface phagocytosis is clearly shown in the tests performed on moistened filter paper and on the fresh tissues.

When the virulence of all four strains in rats is compared to their susceptibility to phagocytosis by rat leucocytes in vitro (table 3), the lack of correlation between virulence and phagocytability on glass slides and in glass roller tubes, as contrasted to the relatively good correlation between virulence and susceptibility to phagocytosis on moistened filter paper, is again evident. In analogous experiments performed with mice the same relationships were demonstrated.

To determine the relative importance of the M protein and the hyaluronic acid capsule as antiphagocytic factors, further experiments were performed with trypsin, to remove the M protein, and with hyaluronidase, to eliminate the capsule

(table 4). The results obtained clearly indicated that *both the M protein and the capsule are involved in protecting the streptococcal cell from phagocytosis, the total antiphagocytic action being due to their combined effects.* But here again, the differences in susceptibility to phagocytosis could only be clearly demonstrated in the tests designed to measure surface phagocytosis.

The finding that virulence is related to surface phagocytosis, as measured in vitro, immediately suggests that this form of phagocytic reaction may play a significant role in the defense of the host against group A streptococcus. Evidence supporting this hypothesis has been obtained by systematic histologic studies of the early stages of streptococcal peritonitis in mice.

The serial peritoneal smears, photographed in figure 4, show that the relatively avirulent strain, T14 (top row of photomicrographs), which is highly susceptible to surface phagocytosis in vitro, was rapidly ingested by both the monocytes and the polymorphonuclear leucocytes of the peritoneal exudate during the first few hours of the infection. Accordingly, it failed to establish a progressive lesion. In contrast, the virulent T14/46 strain (bottom row of photomicrographs), which resists surface phagocytosis, remained out of the leucocytes in sufficient numbers to produce eventually an overwhelming infection. The same comparative results have been obtained with the two S23 strains. Thus there appears to be a significant correlation between virulence and susceptibility to surface phagocytosis both in vitro and in vivo.

It is evident from these experiments that the principal differences in phagocytability which relate to pathogenicity are adequately demonstrated in vitro only by the phagocytic tests specifically designed to measure surface phagocytosis. This fact, supported by the in vivo observations just described, strongly suggests that surface phagocytosis plays an important role in the natural defense of the host against group A streptococci, particularly during the initial stages of infection.

The situation regarding staphylococcal infections appears to be somewhat different and more complicated. Virulent coagulase-positive staphylococci have been shown by Cohn and Morse (1959) to be more resistant to phagocytosis than avirulent coagulase-negative strains, when tested in the absence of antibody. In addition, as demon-
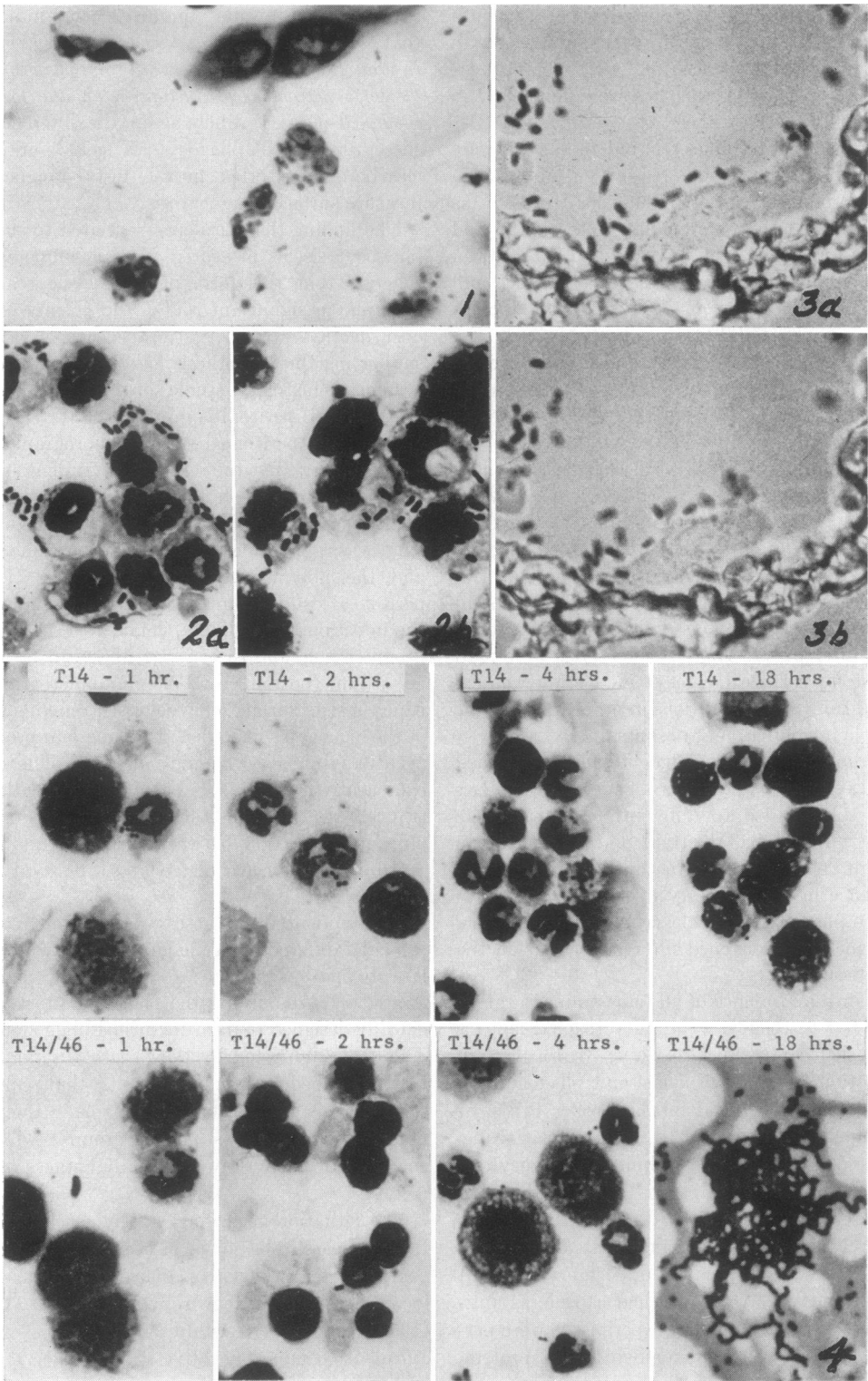
TABLE 1

Comparative properties of group A streptococcal strains used in phagocytic experiments

Strain	Type	Size of Capsule	Production of M Protein	LD₅₀ Mice*	Log LD₅₀ Mice†	LD₅₀ Rats*	Log LD₅₀ Rats†
S23M	14	+++	+++	1	0	1.0×10^3	3.0181
S23G	14	+++	0	1.4×10^5	5.1610	3.6×10^6	6.5481
T14/46	14	++	+++	1	0	2.1×10^5	5.3321
T14	14	++	++	1.4×10^7	7.1400	5.8×10^8	8.7672

* Expressed in number of streptococcal units injected intraperitoneally (6 animals per dilution) and calculated according to the Reed-Muench method.

† Standard error for each log LD_{50} mice is, in order, as follows: ±0.44, ±0.52, ±0.34, ±0.34; and for each log LD_{50} rats is: ±0.55, ±0.35, ±0.51, ±0.28.

strated by Rogers and Tompsett (1952), a small, but demonstrable, fraction of phagocytized co-agulase-positive staphylococci is capable of surviving and multiplying within polymorphonuclear leucocytes. The observations to date, therefore, indicate that the virulence of staphylococci may depend both upon their resistance to phagocytosis and the ability of at least a few of them to survive within phagocytic cells.

Cohn and Morse (1959) have also obtained evidence that the antiphagocytic properties of virulent staphylococci are due to a surface component of the staphylococcal cells. Whether a definite capsule is involved, of the type first described by Lyons (1937) and later noted by Price and Kneeland (1956), remains to be determined. The failure of earlier workers to demonstrate the resistance of virulent staphylococci to phagocy-tosis undoubtedly stems from the fact that practically all of the tests were performed with human serum, which regularly contains staphylococcal antibodies (Jensen, 1958).

The presence of staphylococcal antibodies in human sera suggests that acquired specific immunity plays a role in the natural resistance of man to staphylococcal diseases. Nonspecific immune factors, however, may also be involved. Certainly, in animals such as rabbits, which often have no demonstrable staphylococcal antibody in their sera (Cohn and Morse, 1959), nonspecific mechanisms of defense must operate. The question as to whether surface phagocytosis plays a part in this nonspecific immunity has not been studied. It is quite possible that the virulent strains of staphylococci, which were shown by Cohn and Morse to resist phagocytosis in the test tube, will be

Figure 1. Phagocytosis of pneumococci by polymorphonuclear leucocytes in footpad of rat 1 hr after inoculation (×1440).

Figure 2a. Failure of leucocytes to phagocyte unopsonized Friedländer's bacilli on glass slide (×1300).

Figure 2b. Phagocytosis of Friedländer's bacilli resulting from incubation of same suspension on moistened filter paper (×1300).

Figure 3a. Surface phagocytosis of Friedländer's bacilli in thin section of normal rat lung. The fluid medium in which the leucocytes and the bacteria were suspended consisted of gelatin-Locke's solution. The lung was fixed for 24 hr in 10 per cent formalin and was washed for several days in tap water to remove the formalin before being sectioned and mounted on a cover slip. A drop of the leucocyte-bacillus mixture was then placed on the cover slip which was inverted on a hollow ground slide and placed in a warm stage (37°C) under the microscope. A leucocyte moving down the alveolar wall (from right to left) can be seen trapping an encapsulated bacillus against the alveolar wall of the fixed lung. Note the two delicate finger-like pseudopods between which the organism is being caught (×1500).

Figure 3b. The same cell a moment later in the process of ingesting a second bacillus. The first organism can be seen in the cytoplasm of the leucocyte just above and to the right of the pseudopod which is pinning the second bacillus against the wall of the alveolus (×1500).

Figure 4. Smears of peritoneal exudates made at intervals of 1, 2, 4, 12 and 18 hr during course of peritoneal infections produced in mice with T14 (avirulent) and T14/46 (virulent) strains of group A β-hemolytic streptococci. The T14 infection (*upper*) rarely killed the mice, whereas the T14/46 infection (*lower*) was uniformly fatal. Note the phagocytosis of the T14 organisms by both the monocytes and the polymorphonuclear leucocytes during the early stages of the infection (×1100).

TABLE 2

Susceptibility of strains S23M and S23G to phago-
cytosis by rat leucocytes on glass slides, in glass
roller tubes, on moistened filter paper, and on
surfaces of freshly excised rat tissues

Strain	Phagocytosis*					
	Glass slide	Glass roller tube	Filter paper	Liver	Spleen	Peritoneum
	%	%	%	%	%	%
S23M	0	0	1	0	0	0
S23G	0	0	28	17	26	37

* Percentage of polymorphonuclear leucocytes containing one or more streptococcal units.

TABLE 3

Relation of virulence of streptococcal strains in rats
to their susceptibility to phagocytosis by rat leuco-
cytes on moistened filter paper, on glass slides,
and in glass roller tubes

Strain	LD$_{50}$ Rats*	Phagocytosis†,‡		
		Filter paper	Glass slide	Roller tube
		%	%	%
S23M	1.0×10^3	3	1	1
S23G	3.6×10^6	28	1	0
T14/46	2.1×10^5	10	1	2
T14	5.8×10^8	49	2	2

* As in table 1.

† As in table 2.

‡ Standard deviation (a measurement of the variability of the individual experiments) is for each experiment reading down the 3 columns in order, as follows: 1.8, 3.5, 0.53, 8.9; 0.58, 0.73, 0.62, 0.55; 0, 0, 0.60, 1.0.

found to be susceptible to surface phagocytosis. Such a finding would perhaps help to account for the natural antistaphylococcal resistance of animals which do not possess antibodies in their blood sera.

Finally, a brief comment should be made on methodology. The principal problem involved in studying phagocytosis *in vitro* concerns the maintenance of conditions sufficiently similar to those which obtain *in vivo* to allow a meaningful interpretation of the results obtained (Wood, 1951; Smith and Wood, 1958). The experiments reviewed in the present discussion, for example, demonstrate that in the absence of antibody,

TABLE 4

Effect of trypsin and hyaluronidase on phagocytosis
of strains S23M and S23G

Strain S23M

Treatment	State of capsule*	State of M protein*	Phagocytosis†,‡		
			Filter paper	Glass slide	Roller tube
			%	%	%
None	+++	+++	3	1	1
Trypsin	+++	−	49	1	3
Hyaluronidase	±	+++	41	4	8
Trypsin plus hyaluronidase	±	−	64	29	40

Strain S23G

None	+++	−	28	1	0
Trypsin	+++	−	24	1	3
Hyaluronidase	±	−	74	25	58
Trypsin plus hyaluronidase	±	−	68	43	57

* Arbitrarily designated in terms of + and − symbols. In case of capsule, number of plus signs refers to approximate size of capsular envelope, and ± sign indicates removal of practically all of the capsule by hyaluronidase. The number of plus signs in the M protein column refers to the relative amount of the antigen demonstrable by precipitin test, and a negative sign denotes that none is detectable.

† Percentage of rat polymorphonuclear leucocytes containing one or more streptococcal units.

‡ Standard deviation (a measurement of the variability of individual experiments) is for each experiment, reading down the 3 columns in order, as follows: 1.8, 5.4, 2.8, 0.85, 3.5, 1.1, 3.6, 6.1; 0.6, 1.0, 1.9, 8.5, 0.73, 0.71, 16.0, 5.6; 0, 0, 4.1, 9.0. 0, 0.71, 7.0, 0.

phagocytosis on glass is a very different process from phagocytosis in tissues. Only when the leucocytes are provided with suitable surfaces upon which to operate will they behave as they do *in vivo*. To give them access only to the surfaces of glass slides and test tubes puts them at a serious disadvantage. Also, if they are suspended in mixtures, which are significantly more dilute than inflammatory exudates, their phagocytic powers are greatly reduced (Smith and Wood, 1958). Furthermore, their over-all effectiveness as phag-

ocytes will be influenced by the nature of the medium in which they are suspended. Not only must the medium be such as to preserve the functional integrity of the leucocytes, but it must also contain any humoral factors (including, perhaps, complement), which may promote or accelerate phagocytosis (Ward and Enders, 1933) in inflammatory exudates. Such natural opsonins may well exist and may play a significant role in nonspecific resistance. Thus far, they have not been adequately studied. In the experiments cited dealing with surface phagocytosis, the leucocytes were suspended in gelatin-Locke's solution. This medium was used to exclude the presence of specific antibody. Experiments are now in progress in which serum devoid of specific opsonins is being used as the suspending medium. It will be of interest to determine whether surface phagocytosis in the presence of serum is even more efficient than hitherto demonstrated.

Regardless of the outcome of these unfinished experiments, it is already clear that surface phagocytosis plays an important role in the nonspecific resistance of the host, particularly to infections caused by encapsulated bacteria.

In conclusion, it should be emphasized that the phagocytic activities of granulocytes, monocytes, and the fixed macrophages of the reticulo-endothelial system may be affected by a wide variety of agents including: ionizing radiation (Gordon and Miller, 1955), bacterial endotoxins (Benacerraf et al., 1959), steroid hormones (Kass and Finland, 1953), and viruses (Fisher and Ginsberg, 1956). By influencing the effectiveness of the phagocytic defense, all of them may alter nonspecific resistance to infection. Elucidation of the precise mechanisms by which these agents act is likely to require experiments performed in vitro. If the results obtained in such experiments are to be meaningful in terms of phagocytosis in vivo, the variables emphasized in the present review will have to be controlled.

REFERENCES

BENACERRAF, B., SEBESTYEN, M. M., AND SCHLOSSMAN, S. 1959. A quantitative study of the kinetics of blood clearance of P³²-labeled Escherichia coli and staphylococci by the reticulo-endothelial system. J. Exptl. Med., 110, 27–48.

BERTHRONG, M. AND CLUFF, L. E. 1953 Studies on the effect of bacterial endotoxins on rabbit leucocytes. I. Effect of intravenous injection of the substances with and without induction of the local Shwartzman reaction. J. Exptl. Med., 98, 331–348.

BRAUDE, A. I., CAREY, F. L., AND ZOLESKY, M. 1955 Studies with radioactive endotoxin. II. Correlation of physiological effects with distribution of radioactivity in rabbits injected with lethal doses of E. coli endotoxin labelled with radioactive sodium chromate. J. Clin. Invest., 34, 858–866.

BUCHNER, H. 1890 Untersuchungen über die bacterienfeindlichen Wirkunges des Blutes und Blutserums. Arch. Hyg., 10, 84–101.

BULLOCH, W. 1938 History of doctrines on immunity. In The history of bacteriology, pp. 255–283. Oxford Univ. Press, London.

BURROWS, T. W. 1955 Mechanisms of microbial pathogenicity. The basis of virulence for mice of P. pestis. Symposium Soc. Gen. Microbiol., 5, 152–175.

COHN, Z. AND MORSE, S. I. 1959 Interaction between rabbit polymorphonuclear leucocytes and staphylococci. J. Exptl. Med., 110, 419–443.

COLLINS, R. D. AND WOOD, W. B. 1959 Studies on the pathogenesis of fever. VI. The interaction of leucocytes and endotoxin in vitro. J. Exptl. Med., 110, 1005–1016.

DUBOS, R. J. 1945a The bacterial cell, p. 252. Harvard Univ. Press, Cambridge, Mass.

DUBOS, R. J. 1945b The bacterial cell, pp. 205–212. Harvard Univ. Press, Cambridge, Mass.

FISHER, T. N. AND GINSBERG, H. S. 1956 The reaction of influenza virus with guinea pig polymorphonuclear leucocytes. III. Studies on the mechanism by which influenza viruses inhibit phagocytosis. Virology, 2, 656–664.

FLOREY, H. W. 1958 Chemotaxis, phagocytosis and the formation of abscesses. In General pathology, pp. 67–97. W. B. Saunders Co., Philadelphia.

FOLEY, M. J. AND WOOD, W. B. 1959 Studies on the pathogenicity of group A streptococci. II. The antiphagocytic effects of the M protein and the capsular gel. J. Exptl. Med., 110, 617–628.

FOLEY, M. J., SMITH, M. R., AND WOOD, W. B. 1959 Studies on the pathogenicity of group A streptococci. I. Its relation to surface phagocytosis. J. Exptl. Med., 110, 606–616.

GEY, G. O. 1954 Some aspects of the constitution and behavior of normal and malignant cells maintained in continuous culture. Harvey Lectures, Ser. 50, 167–185.

GORDON, L. E. AND MILLER, C. P. 1955 Clearance of bacteria from blood of irradiated rabbits. Federation Proc., 14, 404.

HIRSCH, J. G. 1958 Bactericidal action of histones. J. Exptl. Med., **108**, 925–944.

JENSEN, K. 1958 A normally occurring staphylococcus antibody in human sera. Acta Pathol. Microbiol. Scand., **44**, 421–428.

KASS, E. H. AND FINLAND, M. 1953 Adrenocortical hormones in infection and immunity. Ann. Rev. Microbiol., **7**, 361–388.

LYONS, C. 1937 Antibacterial immunity to *Staphylococcus pyogenes*. Brit. J. Exptl. Pathol., **18**, 411–422.

MACKANESS, G. B. 1952 The action of drugs on intracellular tubercle bacilli. J. Pathol. Bacteriol., **64**, 429–446.

MACLEOD, C. M. 1958a The pneumococci. In *Bacterial and mycotic infections of man*, pp. 239–242. J. B. Lippincott Co., Philadelphia.

MACLEOD, C. M. 1958b Pathogenic properties of bacteria and defense mechanisms of the host. In *Bacterial and mycotic infections of man*, p. 88. J. B. Lippincott Co., Philadelphia.

MAGOFFIN, R. L. AND SPINK, W. W. 1951 The protection of intracellular brucella against streptomycin alone and in combination with other antibiotics. J. Lab. Clin. Med., **37**, 924–930.

MCCARTY, M. 1958 The hemolytic streptococci. In *Bacterial and mycotic infections of man*, pp. 253–256. J. B. Lippincott Co., Philadelphia.

METCHNIKOFF, E. 1887 Sur la lutte des cellules de l'organismes contre l'invasion des microbes. Ann. inst. Pasteur, **1**, 321–326.

PRICE, K. M. AND KNEELAND, Y. 1956 Further studies of the phenomenon of capsular swelling of *Micrococcus pyogenes* var. *aureus* in the presence of immune serum. J. Bacteriol., **71**, 229–230.

ROGERS, D. E. AND TOMPSETT, R. 1952 The survival of staphylococci within human leucocytes. J. Exptl. Med., **95**, 209–230.

ROUS, P. AND JONES, F. S. 1916 The protection of pathogenic microorganisms by living tissue cells. J. Exptl. Med., **23**, 601–612.

SALE, L. AND WOOD, W. B. 1947 Studies on the mechanism of recovery in pneumonia due to Friedländer's bacillus. I. The pathogenesis of Friedländer's bacillus pneumonia. J. Exptl. Med., **86**, 239–247.

SALE, L., SMITH, M. R., AND WOOD, W. B. 1947 Studies on the mechanism of recovery in pneumonia due to Friedländer's bacillus. II. The effect of sulfonamide chemotherapy upon the pulmonary lesion of experimental Friedländer's bacillus pneumonia. J. Exptl. Med., **86**, 249–256.

SAWYER, W. D., SMITH, M. R., AND WOOD, W. B. 1954 The mechanisms by which macrophages phagocyte encapsulated bacteria in the absence of antibody. J. Exptl. Med., **100**, 417–424.

SHAFFER, J. M., KUCERA, C. J., AND SPINK, W. W. 1935a The protection of intracellular brucella against therapeutic agents and the bactericidal action of serum. J. Exptl. Med. **97**, 77–89.

SHAFFER, J. M., KUCERA, C. J., AND SPINK, W. W. 1953b Evaluation of prolonged antibiotic therapy in mice with chronic brucella infection due to *Brucella melitensis*. J. Immunol., **70**, 31–38.

SMITH, M. R. AND WOOD, W. B. 1947 Studies on the mechanism of recovery in pneumonia due to Friedländer's bacillus. III. The role of "surface phagocytosis" in the destruction of the microorganisms in the lung. J. Exptl. Med., **86**, 257–266.

SMITH, M. R. AND WOOD, W. B. 1949a Relation of surface phagocytosis to the fibrinous character of acute bacterial exudates. Science, **110**, 187–188.

SMITH, R. O. AND WOOD, W. B. 1949b Cellular mechanisms of antibacterial defense in lymph nodes. I. Pathogenesis of acute bacterial lymphadenitis. J. Exptl. Med., **90**, 555–566.

SMITH, M. R. AND WOOD, W. B. 1958 Surface phagocytosis. Further evidence of its destructive action upon fully encapsulated pneumococci in the absence of type-specific antibody. J. Exptl. Med., **107**, 1–12.

STEVENS, K. M. AND MCKENNA, J. M. 1958 Studies on antibody synthesis initiated *in vitro*. J. Exptl. Med., **107**, 537–559.

SUTER, E. 1956 Interaction between phagocytes and pathogenic microorganisms. Bacteriol. Revs., **20**, 94–132.

VON BEHRING, E. AND KITASATO, S. 1890 Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. Deut. med. Wochschr., **16**, 1113–1114.

WARD, H. AND ENDERS, J. F. 1933 An analysis of the opsonic and tropic action of normal and immune sera based on experiments with the pneumonoccus. J. Exptl. Med., **57**, 527–547.

WOOD, W. B. 1951 Studies on the cellular immunology of acute bacterial infections. Harvey Lectures, Ser. **47**, 72–98.

WOOD, W. B. AND SMITH, M. R. 1947 Intercellular surface phagocytosis. Science, **106**, 86.

WOOD, W. B., MACLEOD, C., AND IRONS, E. N. 1946a Studies on the mechanism of recovery in pneumococcal pneumonia. II. Factors influencing the phagocytosis of pneumococci in the lung during sulfonamide therapy. J. Exptl. Med., **84**, 377–386.

WOOD, W. B., SMITH M. R., AND WATSON, B.

1946*b* Studies on the mechanism of recovery in pneumococcal pneumonia. IV. The mechanism of phagocytosis in the absence of antibody. J. Exptl. Med., **84,** 387–402.

WOOD, W. B., SMITH, M. R., PERRY, W. D., AND BERRY, J. W. 1951 Studies on the cellular immunology of acute bacteremia. I. Intravascular leucocytic reaction and surface phagocytosis. J. Exptl. Med. **94,** 521–534.

WRIGHT, A. E. AND DOUGLAS, S. R. 1903 An experimental investigation of the role of the blood fluids in connection with phagocytosis. Proc. Roy. Soc. (London), **72,** 357–370.

ZINSSER, H., ENDERS, J. F., AND FOTHERGILL, L. 1939 The phenomena of phagocytosis. In *Immunity, principles and applications in medicine and public health*, pp. 317–319. Macmillan Co., New York.

DISCUSSION

(Papers by W. B. Wood and D. Rogers are discussed together. See at end of Rogers' paper.)